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Research article

Involvement of small heat shock proteins (sHsps) in developmental stages of fall armyworm, *Spodoptera frugiperda* and its expression pattern under abiotic stress condition

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ABSTRACT

Fall armyworm (FAW), *Spodoptera frugiperda* a recent invasive pest in India is reported to cause significant damage by feeding voraciously on maize and other economic crops from tropical to temperate provinces. It is becoming an arduous challenge to control the pest as it can survive in a wide range of temperature conditions and is already said to develop resistance towards certain insecticides. The small Heat shock proteins (hereafter, sHsps) are known to play an important role in adaptation of insects under such stress conditions. Our present study involved characterization of the three sHsps genes (sHsp19.74, sHsp20.7 and sHsp19.07) which encoded proteins of about 175, 176 and 165 amino acids with a conserved α-crystalline domain. Phylogenetic analysis of deduced amino acid sequences of the three genes showed strong similarity with the other lepidopteran sHsps. The effect of different growth stages on the expression profile of these stress proteins has also been studied and the Quantitative real time PCR (qRT-PCR) analysis revealed that the transcript level of sHsp19.07 and sHsp20.7 were significantly upregulated under extreme heat (44 °C) and cold (5 °C) stress. However, sHsp19.74 responded only to heat treatment but not to the cold treatment. In addition, the expression profile of all three sHsps was significantly lower in the larval stage (5th instar). Chlorantraniliprole treatment resulted in maximum expression of sHsp19.07 and sHsp20.7 after 12hr of exposure to the insecticide. Meanwhile, the same expression was observed after 8hr of exposure in case of sHsp19.74. These results proved that the sHsp genes of *S. frugiperda* were induced and modulated in response to abiotic stress, thus influencing the physiological function leading to survival of FAW in diversified climate in India.

1. Introduction

Heat shock proteins (Hsps) are products of different biotic and abiotic stress responses (Zhao and Jones, 2012). Based on their molecular weight and homologous relationship the Hsps are primarily divided into five families, namely Hsp100, Hsp90, Hsp70, Hsp60, and sHsps (Li et al., 2009). The small heat shock proteins (sHsps), with molecular weights of about 12–43 kDa are probably the most diverse class among various other stress proteins (Franck et al., 2004). Owing to their ubiquitous nature, these sHsps are reported to be present in single celled organisms like algae to higher organisms including humans (Kim et al., 1998; Waters and Rioflorido, 2007). Ten sHsps, namely HspB1-HspB10 have been identified in the human genome (Kappe et al., 2003). Moreover, small heat shock proteins (sHsps) were also reported in marine viruses (cyanophages) (Maaroufi and Tanguay, 2013). Besides functioning as molecular chaperones to protect denaturation of proteins from high temperature stress (Van Montfort et al., 2001; Sun et al., 2014), sHsps respond to other stress conditions including UV, heavy metals and also higher population density of other organisms (Waters et al., 2008; Gu et al., 2012). The C-terminal of these proteins harbors the conserved α-crystalline domain however, the N-terminal sequences are somewhat variable (Li et al., 2009). This indicates an association of diverse N-terminals with differential expression and evolutionary patterns of the stress proteins. The sHsps are evolutionarily related to alpha-crystallin with a

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conserved domain, which is an abundant constituent of the eye lens of most vertebrate species (Augusteyn, 2004).

Studies have been conducted on Hsps in algae, birds, mammals and other model organisms including *Drosophila melanogaster*, *Arabidopsis thaliana*, *Danio rerio* etc (Waters and Rioflorido, 2007; Waters et al., 2008; Aevermann and Waters, 2008). Despite the heat shock response that was first studied in the insect, *D melanogaster* (Ritossa, 1962), the function of Hsp in case of other insects, especially the sHsps is still unexplored in comparison to other organisms. Earlier researches have stated involvement of these stress proteins in heat/cold adaptations, metamorphosis, diapause and several other critical functions in insects (Gu et al., 2012; Hayward et al., 2005; Jakob and Buchner, 1994; Lu et al., 2014; Rinehart et al., 2007; Song et al., 2006). The role of Hsps in regulating insect responses to various insecticides has also been explicitly studied (Dumas et al., 2019). Among the many other functions, participation of sHsps in thermal resistance is considered to be the most important one (Qin et al., 2005; Huang et al., 2009) enabling insects to thrive across extensive thermal regimes (Huang and Kang, 2007). Thermal tolerance of an invasive species is considered to be an important factor for its successful establishment and spread (Kang et al., 2009).

The Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a recent invasive pest in India, first reported from Bangalore in 2018 inflicting serious damage to the maize crop. (Kalleshwaraswamy et al., 2018). Since then this pest has extended its geographical distribution, causing significant damage to many others economically important crop (Swamy et al., 2018). However, the underlying mechanism explaining the sudden outbreak and widespread distribution pattern of this invasive pest remains murky. To gain a clear insight this study was designed to focus on the response of three sHsps in FAW upon subjection to heat, cold and insecticidal stress. The expression profile at different growth stages of the insect was also monitored in parallel. For better understanding of the functional and evolutionary pattern of these stress proteins, we have isolated and characterized the three sHsps in FAW. Hence, our work reinforces potential importance of sHsps in the newly invasive pest FAW and provides insights into evolution and function of these stress proteins.

2. Materials and methods

2.1. Insect rearing

Spodoptera frugiperda were collected from corn grown in the university research farm and raised at the laboratory, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, India. Briefly, cultures were raised in 10 × 6 × 4 inches rectangular box covered with fine mesh cloth. Insects were fed on fresh corn leaves and kept under controlled environmental condition at 26 ±1 °C with 60% R.H and 16 h light/8h dark condition. Two generations were raised prior to experiment.

2.2. Sample preparation

For studying the expression pattern of three sHsps under different temperature condition the late larval stage (5^{th} instar) were exposed to cold (5 °C and 15 °C), heat (35 °C and 44 °C) shock for 1 h, and then maintained at room temperature (26 °C) before RNA extraction. Larvae kept at 26 °C was considered as control.

Different stages of FAW were collected separately and subjected to RNA extraction for studying expression pattern of three sHsp at different developmental period. The stages were defined as: EE (1 day after oviposition, early egg), LL (5^{th} instar larvae, late larvae), EP (1 day old, early pupa), EA (24h after emergence, early adult). Insecticide treatment of FAW was carried out by placing the insects in an incubator at 26 °C for 2 days under 16 h light and 8h dark condition (Growth chamber G-1000, S. Korea). The solution of chlorantraniliprole 18.5 SC (Coragen 200 g/L Soluble Concentrate) was prepared in water and sprayed topically on the abdomen of 10 larvae's and equal amount of water was sprayed on 10 larvae in a similar way which was considered as control. The expression of three sHsps was observed at 4hr, 8hr and 12hr after insecticidal spray. The above treated samples were frozen immediately in liquid nitrogen and kept at -80 °C for downstream analysis.

2.3. Molecular identification, RNA isolation, cDNA synthesis and gene cloning

Molecular identification of FAW was confirmed by using Mtco1 gene specific primers following the protocol given by Hajibabaei et al., 2006, the sequence was then submitted to EMBL (Accession no. PRJEB41471). RNA was extracted from the abdomen of treated larvae by using Insect RNA Isolation Kit (Thermo Fisher Scientific) following manufacturer's protocol (Morin et al., 2017a). RNA quality was evaluated using Invitrogen ™ Qubit ™ 4 Fluorometer (Thermo Fisher Scientific) to determine the quality and quantity with high precision per µl of RNA. The First Strand cDNA Synthesis Kit (Genetix Biotech Asia Pvt. Ltd.) was used to isolate cDNA from 1µg total RNA as recommended, and cDNAs were stored at -20 °C until for further use. The primers used in the experiment were designed from the conserved gene sequences of *S. frugiperda* and other closely related insects like *Spodoptera litura*, *Spodoptera exigua* and *Helicoverpa armigera*, retrieved from NCBI Genebank (Table 1). PCR program was carried out in a total volume of 25µl containing 2 µl of Template DNA, 12.5 µl PCR Master Mix, 8.5 µl Molecular Grade Water and 1 µl each Forward and Reverse Primer. Thermal cycler programmed a denaturation at 94 °C for 5 min, followed by 40 cycle of 94 °C for 30 s, annealing at 54 °C for 30 s. Extension was carried out at 72 °C for 40 s with a final extension at 72 °C for 5 min. The purified PCR amplified products were cloned by ligating to the pJET1.2/blunt cloning vector and then transformed into *Escherichia coli* strain DBH10 with the help of bacterial transformation kit (Fermentus, Transform Aid, catalogue No. k2710). The cloned fragments were sent for DNA sequencing by Sanger dideoxy sequencing to Chromus Biotech, India.

2.4. Sequence alignment and secondary structure prediction

The open reading frames (ORFs) of the nucleotide sequences, molecular weight and Isoelectric point of the deduced amino acids of the three gene sequences were identified with the aid of the ORF Finder software (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), Expasy (https://web.expasy.org/protparam/). Multiple-alignment of the amino acid sequences of *S. frugiperda* Hsps was generated using BioEdit sequence alignment software (version 7.2.5) to obtain the clustal consensus sequence (Hall 1999). Secondary structure predictions was performed with PSIPRED Software (http://bioinf.cs.ucl.ac.uk/psipred).PfAM (Pfam version 33.1)was used to identify the conserved domain and predicted superfamily.

2.5. Phylogenetic analysis

Phylogenetic analysis was conducted using three sHsps of FAW along with orthologous amino acids of different orders of insects retrieved from the NCBI website (http://blast.ncbi.nlm.nih). Amino acids of the selected sHsps were aligned using ClustalW and then the phylogenetic analysis was performed with MEGA version X (Kumar et al., 2018). The tree was constructed using neighbor-joining (NJ) with 1,000 bootstrap replications and evolutionary distance was computed using p-distance method.

2.6. Quantitative real-time PCR (qRT-PCR)

The expression pattern of sHsps genes was examined using qPCR protocol. 2X SYBR Green qPCR Master Mix (Applied Biosystems and KAPA Biosystems, USA) was used. Primers name, their sequences have been mentioned in Table 1. The cDNA samples were run in triplicate to ensure validity of the data using Agilent Technologies Stratagene Mx3000P Sequence Detection System. Amplification was carried out

Table 1. Primers used in the current study. The primer name, PCR type, Primer sequences and the Annealing temperature are listed in the table above.

Primer name	PCR type	Primer sequences (5′→3′)		Annealing temperature (°C)
		Primer forward	Primer reverse	
FAW Mt-Co1	Conventional PCR	ATTCAACCAATCATAAAGATATTGG	TAAACTTC TGGATGTCCAAAAAATCA	54
sHsp19.74	RT-PCR	ATTGAAGGAAAGCACGAGGA	CCTACTTGTCGCCATTAGCC	55
sHsp20.7	RT-PCR	CCGCAAGACCAGTTAACCAT	GATGAATCCGTGCTGATCCT	60
sHsp19.07	RT-PCR	GTTTTTGAGGGACCCGTTCT	ACCGTGATCTCGTTTCCAAC	60
sHsp19.74	qRT-PCR	GGGTATATTTCCCGCCAGTT	CTGGTCCTTGACTTCCTTGC	60
sHsp20.7	qRT-PCR	AGCCAAGCATGAGGAGAAGA	CTGGTCCTTGATCTCCTTGC	60
sHsp 19.07	qRT-PCR	CCCGTTCTTCAGAGATCCAG	ACCGTGATCTCGTTTCCAAC	60
*EF	qRT-PCR	TCGCTGTGGGTGTAATCAAG	GCTACTTCTTGCCCTTGGTG	60

* Elongation Factor.

in 10 μl reaction containing 5μl 2X SYBR Green PCR Master Mix, 0.5 μl of each primer (10μM each), 1μl template DNA, 0.2 μl ROX and 2.8μl Molecular Grade Water. The cycling condition were as follows: 3 min activation at 95 °C followed by 40 cycle of 40 s at 95 °C, 40 s at 60 °C and 45 s at 72 °C. The relative expression of each target was calculated by $2^{-\Delta\Delta Ctn}$method (Pusag et al., 2012). As an endogenous control, the expression of EF (Elongation factor) gene was run in parallel.

2.7. Data analysis

The differences in relative expression of sHsps genes under different stress condition were analysed using one way Analysis of variance (ANOVA). The means were compared by Tukey test at $P < 0.05$. The statistical analysis was performed using SPSS 14.0 (SPSS Inc. Chicago, IL).

3. Results

3.1. Molecular characterization of three sHsps

Three small Heat shock genes namely sHsp19.74, sHsp20.74 and sHsp19.07, (GenBank accession nos. MW364359, MW349130 and MW364360 respectively) were obtained from the NCBI. The ORFs obtained, were 498, 528 and 531bp in length respectively, molecular weight of the amino acids of these sHsps were19.71, 20.17 and19.07kDa and isoelectric points were of 6.60, 6.08, 6.60respectively. The protein domains of the deduced amino acids showed significant match with Hsp20/alpha crystallin family. The α-crystal domain of all the three sHsps is composed of approximately ~96 amino acids and 7- βstrand. However, N-terminal end of sHsp19.74 and sHsp19.07 comprised of 1 α helix but arranged in different orders (Figure 1). The C-terminal end of the protein is quite conserved among the three sHsps.

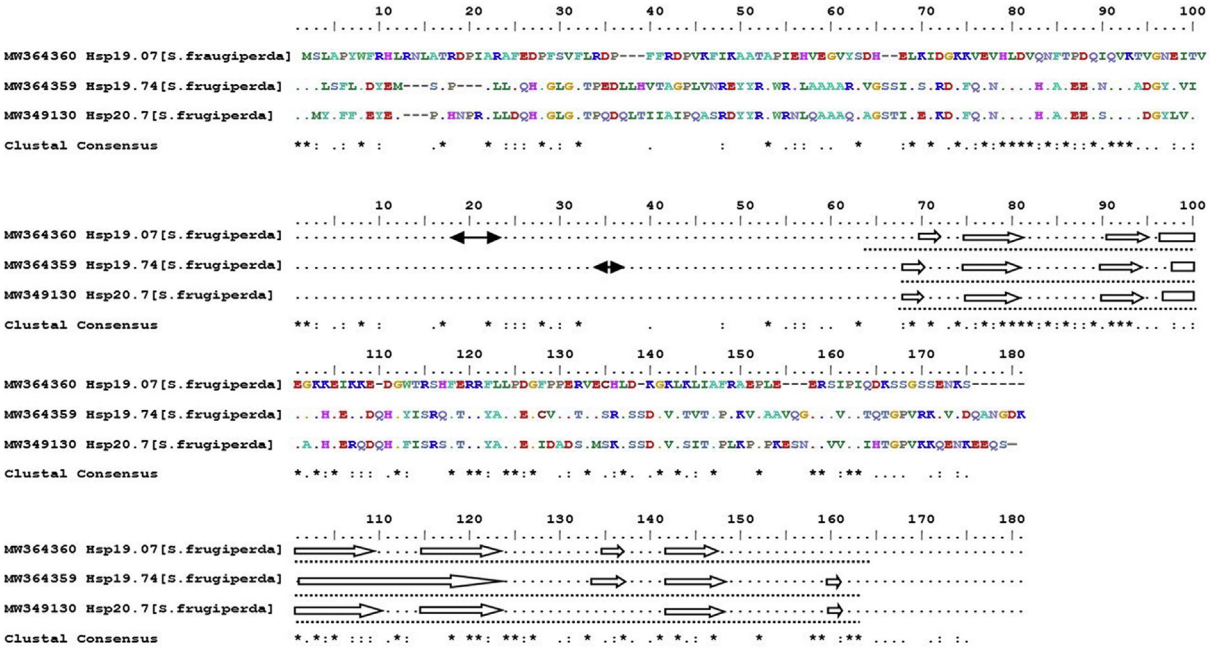

Figure 1. Alignment of the deduced amino acid sequences of three sHsps of FAW. The conserved ά-crystallin domain is underlined. Secondary structure elements are predicted for individual sHsp and are shown by two-sided arrow (ά-helices) and one-sided arrow (β-strands). "*" indicate perfect alignment. ":"indicate a site belonging to group exhibiting strong similarity. "." indicate a site belonging to group exhibiting weak similarity.

Based on the phylogenetic analysis of various sHsps among different insect orders, the three sHsps were assigned into two different lepidopteran cluster groups. sHsp19.74 (MW364359) and sHsp20.7 (MW349130) were under one big lepidopteran cluster. sHsp19.74 belonged to the sister clade of sHsp19.7 (*S.litura*) and sHsp19.74 (*S.frugiperda*). Whereas sHsp20.7 (MW349130) was found to be congregated under a different clade within sHsp20.7 (*S. litura* and *S. frugiperda*), and sHsp20.1 (*S.frugiperda*). On the other hand sHsp19.07 shared its sister clade with sHsp19.5 *Helicoverpa armigera* and *Mythimna seperata* (Figure 2).

3.2. Expression of FAW sHsps in response to temperature stress

The transcript level of the three sHsps varied when subjected to different temperature treatment. At extreme high temperature i.e. 44 °C, transcript levels of sHsp19.07, sHsp20.7 and sHsp19.74 were significantly up regulated upon subjection to heat ($F_{3,8} = 4.34$, $P = .04$, $F_{3,8} = 10.32$, $P = .004$ and $F_{3,8} = 44.82$, $P = .0001$ respectively) with increases of 2.93, 1.42 and 1.41 fold respectively (Figure 3). At 35 °C, the transcript level of sHsp19.07 and sHsp20.7 showed maximum expression with increases of 79.44 and 59.44 fold respectively. However, at same temperature treatment the transcript level of sHsp19.74 displayed significant reduction with values of 0.38 fold. At 15 °C the expression of all three sHsp i.e. sHsp19.07, sHsp20.7 and sHsp19.74 were downregulated with decreases of 0.15, 0.11 and 0.54 fold respectively. At extreme low temperature (5 °C) the expression levels of sHsp19.07 and sHsp20.7 increased by 56.84 and 22.36 fold with marked decrease of 0.08 fold in transcript level of sHsp19.74.

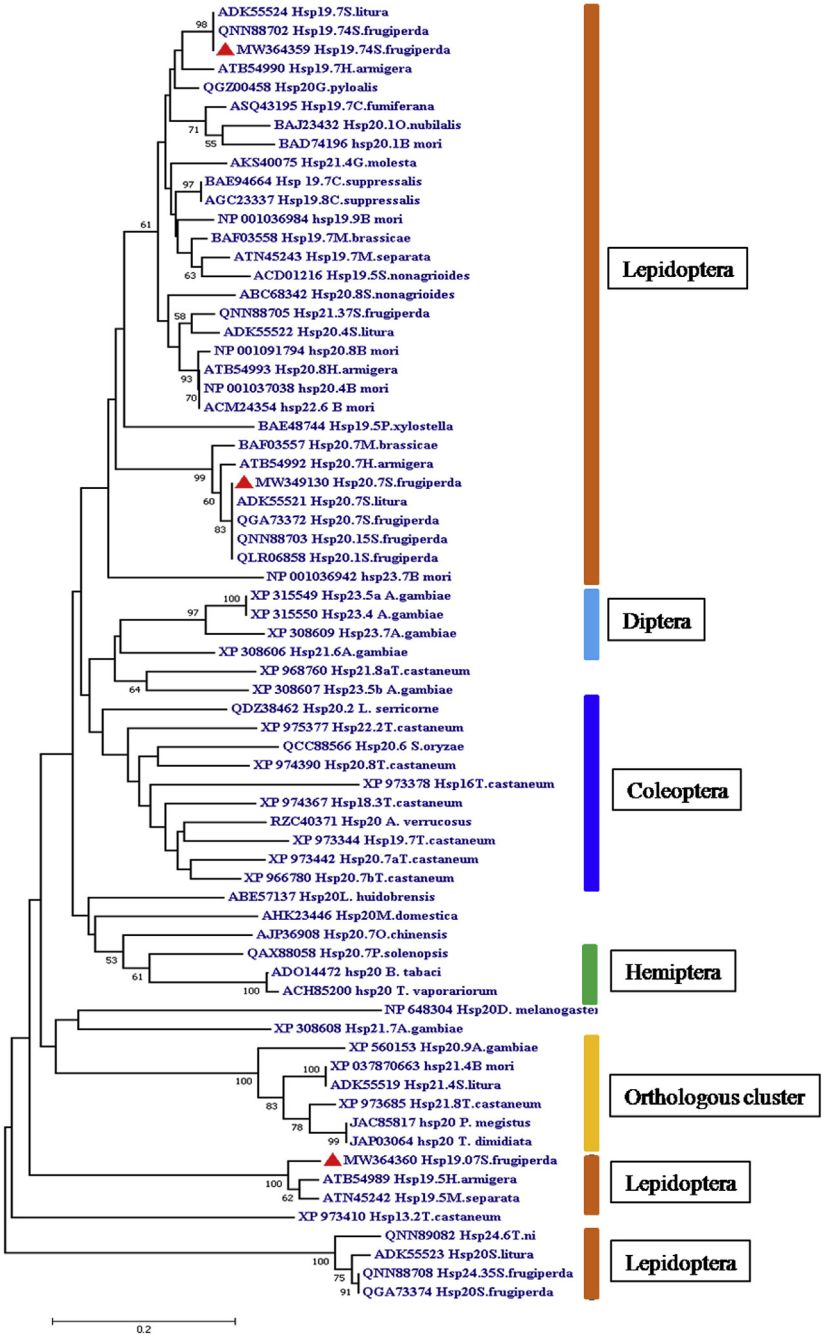

Figure 2. Phylogenetic analysis of sHsps. A total of 69sHsps of different insect orders (Order Name and GenBank accession numbers provided) were selected to construct the evolutionary phylogenetic tree. The evolutionary tree was constructed using Neighbour-joining method using MEGA X. The optimal tree with sum of branch length is 3.2598. The evolutionary distance was calculated using p-distance method. Percentage bootstrap was conducted in 1000 replicates, where the percentage bootstrap above 50% was indicated next to the branches. The three sHsps of *S. frugiperda* are labeled in red triangle.

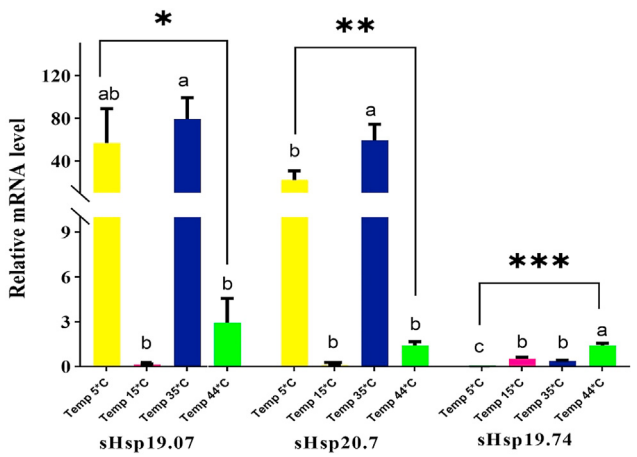

Figure 3. Effect of cold (5̊ C and 15̊ C) and heat shock (35̊ C and 44̊ C) treatments on the transcript level of sHsp19.07, sHsp20.7 and sHsp19.74 in the late larval stage of FAW (5th instar). Larvae exposed to 26̊ C was considered as control. Relative levels of each sHsps were measured by qRT-PCR with EF acting as a reference gene. The different letters indicate statistically significant differences between the treatments. P ≤ 0.05 is indicated by *, P ≤ 0.01 is indicated by ** and P ≤ 0.001 is indicated by ***.

3.3. Expression of FAW sHsps at different developmental stages

The expression pattern of the threes sHsps were significantly different among the growth stages in FAW (Figure 4). Expression level of both sHsp20.7 and sHsp19.74 downregulated in the late larval stage with decreases of 0.07 and 0.03 fold respectively followed with gradual increase in the early pupal and early adult stages. In contrast, sHsp19.07 exhibited lower expression levels in both late larvae (0.76-fold) and early adult stages (0.23-fold) as compared to the early egg and early pupal stages.

3.4. Expression of FAW sHsps after chlorantraniliprole spray at different time interval

mRNA transcript level of sHsp19.74, sHsp19.07 and sHsp20.07 were measured by qRT-PCR following Chlorantraniliprole 18.5 SC treatment in FAW (Figure 5). The concerned genes showed irregular expression patterns with time interval. Transcript levels of sHsp19.07 initially decreased from 0.5 fold to 0.15 fold at 4 hr and 8 hr respectively with gradual elevation to 5.57 fold after 12 hr of treatment when compared to untreated insects ($F_{2,6} = 94.40$, $P = .0001$). In case of sHsp19.74 and sHsp20.07 the expression levels reached their maximum after 8 hr and 12 hr of insecticide treatment with increases of 209.34 and 19.86 fold respectively ($F_{2,6} = 42.11$, $P = .0001$ and $F_{2,6} = 8.15$, $P = .019$).

4. Discussion

The essential roles of sHsps in various metabolic functions of insects such as development, reproduction and adaptation to different biotic and abiotic stress condition have been well documented (Bai et al., 2019; Concha et al., 2012; Takahashi et al., 2010). As a member of heat shock protein family, these sHsps help in defending other proteins from various challenges in the environment by maintaining their normal state (Shi et al., 2013; Zhang et al., 2015; Chen et al., 2016). The critical roles of sHsps in thermal adaptations of insects have been previously reported (Gehring and Wehner, 1995; Huang and Kang, 2007; Huang et al., 2009). In the present study transcript levels of sHsp19.07 and sHsp20.7 were significantly upregulated under extreme heat (44 °C) and cold (5 °C) stress. Induction of these genes to cold stress suggests their involvement in cold adaptation of FAW across such regimes. However, sHsp19.74 was strongly induced by heat stress but not to the cold stress. Our results showed quite good congruence with the findings on Hsp 21.4 (*Bombyx mori)*, Hsp20 and Hsp21.4 (*Spodoptera litura)*, Bthsp19.5, Bthsp19.2, and Bthsp21.3 (*Bemicia tabaci)* (Li et al., 2009; Lu et al., 2014; Bai et al., 2019) where the stress proteins reported to be less responsive to cold. A possible explanation for such differences in heat and cold stress response is a result of differential stimulation of heat shock factor (HSF) isoforms (Fujikake et al., 2005). Some reports further suggested that HSP genes significantly upregulates only during recovery of insect from cold shock and might not be induced during cold stress (Sinclair et al., 2007; Colinet et al., 2010). However, whether the genes that were not induced by cold stress will significantly upregulate during recovery from cold shock will require further investigation. Our results also re-establish the fact that heat and cold adaptation in insects can function separately and independently (Huang and Kang, 2007). Thus, the thermal adaptation of FAW under different temperature regimes may arise from combined expression of these sHsps.

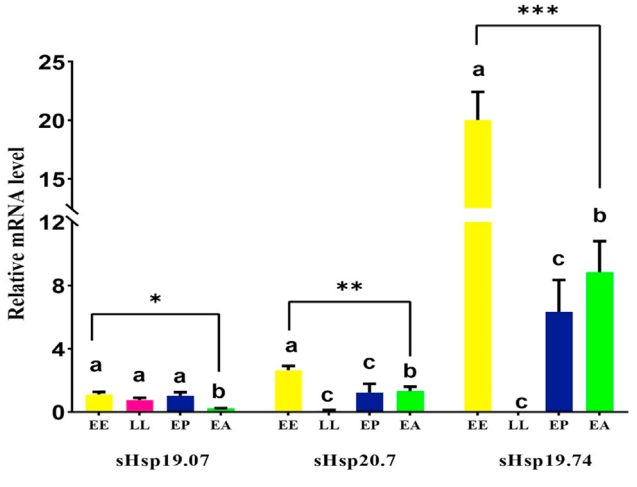

Figure 4. Relative mRNA expression level of three sHsps in different developmental stages of FAW. (Different developmental stages are indicated by EE: early egg; LL: late larvae; EP: Early pupae; EA: Early adult. Relative levels of each sHsp was measured by qRT-PCR with EF acting as a reference gene. The different letters indicate statistically significant differences between the treatments. P ≤ 0.05 is indicated by *, P ≤ 0.01 is indicated by ** and P ≤ 0.001 is indicated by ***.

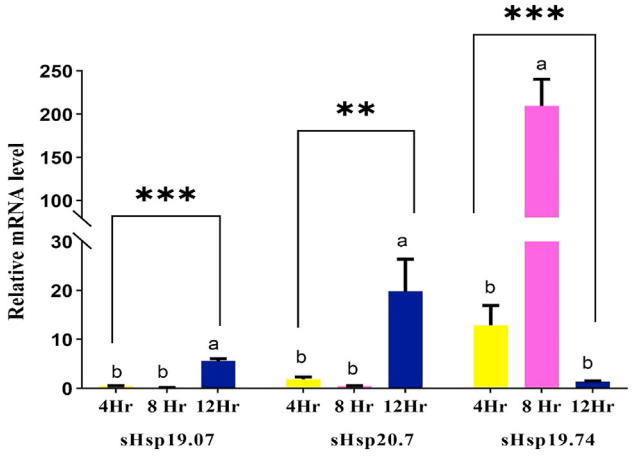

Figure 5. Effects of Chlorantraniliprole 18.5 SC (200 g/L) on transcript levels of sHsp19.07, sHsp20.7 and sHsp19.74 at different time (4Hr, 8Hr and 12Hr) intervals. Different expression levels of sHsps were measured by qRT-PCR with EF as a reference gene. The different letters indicate statistically significant differences between the treatments. P ≤ 0.05 is indicated by *, P ≤ 0.01 is indicated by ** and P ≤ 0.001 is indicated by ***.

The role of small heat shock proteins in insect development is well documented (Takahashi et al., 2010). In insects such as *Plutella xylostella,* Hsp19.7 is expressed at lower levels in both larval and adult stages but upregulated in pupal stage (Sonoda et al., 2006). In *S. litura* SlHsp19.7 and SlHsp20.7 showed lower expression in larval stage with upregulation of transcript level in adult stage (Shen et al., 2011). Similarly, in the present study transcript level of both sHsp19.74 and sHsp20.7 maintained a lower expression in the late larval stage with gradual increase in subsequent stages suggesting its possible involvement in metamorphosis. In case of sHsp19.07, maximum upregulation of expression was found in the early egg stage, which is in accordance with earlier findings where expression of SiHsp20.6 and SiHsp19.6 was highest in the egg stage of *Sesemia inferens* (Sun et al., 2014). sHsp19.07 showed a constitutive expression pattern throughout all the developmental stages. However, in case of sHsp19.74 the transcript level reaches a peak at early egg stage followed by a steep decline at late larval stage. This clearly suggests that sHsps might have evolved different roles at different developmental stages of insects. Lower expression of all the three small heat shock protein in the late larval stage of FAW makes it more vulnerable to temperature and chemical stress hinting towards possible management of this pest at the larval stage.

In recent findings potential importance of Heat Shock proteins in modulating responses of insects to various insecticides have been highlighted. Our work has also emphasised the differential response of sHsps in FAW after exposure to Chlorantraniliprole at different time intervals. The involvement of these sHsps in resistance development against insecticides in FAW has not been reported previously. After chlorantraniliprole treatment, the expression of all the three sHsps showed a time dependent response. The maximum expression of sHsp19.07 and sHsp20.7 was observed after 12 hr of exposure to the insecticide but in the case of sHsp19.74, mRNA expression levels reached their maximum after 8hr of exposure. Hence, it can be stated that when FAW populations are subjected to insecticide treatment, sHsp19.74 responds faster than the other two sHsps indicating its involvement in first line of defense against chemical stress. Another notable factor is that expression level of sHsp19.74 increased significantly after 8 h of exposure to chemical stress and dramatically decreased at 12 h after exposure. This suggests that induction of sHsp can be rapid or transitory in response to insecticide treatment. Studies reveal a decrease in the transcript levels of Hsp90 in *Apis melllifera* when exposed to Imidaclorpid (Derecka et al., 2013). Elevation in transcript levels of Hsp90 were observed in *A. lucorum* when exposed to Cyhalothrin, Imidaclorpid, Chlorpyriphos and Emamectin benzoate (Sun et al., 2014). Lu et al. (2017) reported upregulation in expression of Hsp70 in brown planthopper, *Nilaparvata lugens* when treated with Imidaclorpid. Literature focusing on the time specific response of sHsps upon subjection to chemical stress is very scarce and requires more attention.

The information regarding the secondary structure of sHsps and their domain play a pivotal role in enabling researchers to fathom the function of these stress proteins. Except for the conserved α-crystalline domain, which is located in the C-terminal, the N-terminals are highly variable (Shen et al., 2011). The variable N-terminal sequences may be linked with differential expression and evolutionary pattern of sHsps (Li et al., 2009) and is critical for chaperone activity and substrate specificity. Based on the secondary structure of the three sHsps used in this study, the N-terminals are found to be highly variable with the presence of a α-helix at different positions in case of sHsp19.07 and sHsp19.74 but absence of any α-helix in case of sHsp20.7. Probably, the functional variations of the sHsps are associated with the structural difference in the N-terminal region.

In conclusion, the data presented in this study demonstrate the expression pattern of three sHsps (sHsp19.07, sHsp20.7 and sHsp19.74) of *S frugiperda* when exposed to different temperature and chemical stress. Emphasis is also placed on the role of sHsps in different stages of FAW indicating their involvement in the developmental events of the insect. It is a first time study on the expression of these sHsps in fall armyworm, which might be instrumental in decoding the molecular mechanism behind its wide adaptation ability and response against xenobiotic compounds. As the ability of any invasive pest to tolerate thermal stress is an important determinant of its niche space in new geographical regions, it is of prime importance to focus on the variables associated with this phenomenon. The functional information pertaining to sHsps in this agriculturally important invasive pest in India is lacking. Overall, our work can be considered as an important step towards delineating the role of sHsp in fall armyworm, further addressing the involvement of these sHsps in different developmental and physiological functions of the insect. It is believed that such information might help us understand the adaptability of FAW across different regions of India.

Declarations

Author contribution statement

Snigdha Samanta: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mritunjoy Barman: Conceived and designed the experiments; Performed the experiments.

Swati Chakraborty: Performed the experiments; Analyzed and interpreted the dat.

Amitava Banerjee; Jayanta Tarafdar: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Aevermann, B.D., Waters, E.R., 2008. A comparative genomic analysis of the small heat shock proteins in *Caenorhabditiselegans* and briggsae. Genetica 133, 307–319.

Augusteyn, R.C., 2004. α-crystallin: a review of its structure and function. Clin. Exp. Optom. 87, 356–366.

Bai, J., Liu, X.N., Lu, M.X., Du, Y.Z., 2019. Characterization of genes encoding small heat shock proteins from *Bemisia tabaci* and expression under thermal stress. PeerJ 7, e6992.

Chen, J., Kitazumi, A., Alpuerto, J., Alyokhin, A., de Los Reyes, B., 2016. Heat-induced mortality and expression of heat shock proteins in Colorado potato beetles treated with imidacloprid. Insect Sci. 23, 548–554.

Colinet, H., Lee, S.F., Hoffman, A., 2010. Temporal expression of heat shock genes during cold stress and recovery from chill coma in adult Drosophila melanogaster. FEBS J. 277, 174–185.

Concha, C., Edman, R.M., Belikoff, E.J., Schiemann, A.H., Carey, B., Scott, M.J., 2012. Organization and expression of the Australian sheep blowfly (*Luciliacuprina*) hsp23, hsp24, hsp70 and hsp83genes. Insect Mol. Biol. 21, 169–180.

Derecka, K., Blythe, M.J., Malla, S., Genereux, D.P., Guffanti, A., Pavan, P., Moles, A., Snart, C., Ryder, T., Ortori, C.A., Barrett, D.A., Schuster, E., Stöger, R., 2013. Transient exposure to low levels of insecticide affects metabolic networks of honeybee larvae. PloS One 8, e68191.

Dumas, P., Morin, M.D., Boquel, S., Moffat, C.E., 2019. Expression status of heat shock proteins in response to cold, heat, or insecticide exposure in the Colorado potato beetle *Leptinotarsadecemlineata*. Cell Stress Chaperones 24, 539–547.

Franck, E., Madsen, O., Rheede, T.V., Ricard, G., Huynen, M.A., de Jong, W.W., 2004. Evolutionary diversity of vertebrate small heat shock proteins. J. Mol. Evol. 59, 792–805.

Fujikake, N., Nagai, Y., Popiel, H.A., Kanoa, H., Yamaguchi, M., Todaa, T., 2005. Alternative splicing regulates the transcriptional activity of Drosophila heat shock transcription factor in response to heat/cold stress. FEBS (Fed. Eur. Biochem. Soc.) Lett. 579, 3842–3848.

Gehring, W.J., Wehner, R., 1995. Heat shock protein synthesis and thermotolerance in Cataglyphis, an ant from the Sahara desert. Proc. Natl. Acad. Sci. U. S. A 92, 2994–2998.

Gu, J., Huang, L.X., Shen, Y., Huang, L.H., Feng, Q.L., 2012. Hsp70 and small Hsps are the major heat shock protein members involved in midgut metamorphosis in the common cutworm, Spodoptera litura. Insect Mol. Biol. 5, 535–543.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W., Hebert, P.D.N., 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proc. Natl. Acad. Sci. Unit. States Am. 103, 968–971.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41, 95–98.

Hayward, S.A.L., Pavlidesb, S.C., Tammariellob, S.P., Rineharta, J.P., Denlinger, D.L., 2005. Temporal expression patterns of diapause-associated genes in flesh fly pupae from the onset of diapause through post-diapause quiescence. J. Insect Physiol. 51, 631–640.

Huang, L.H., Kang, L., 2007. Cloning and inter-specific altered expression of heat shock protein genes in two leaf miner species in response to thermal stress. Insect Mol. Biol. 16, 491–500.

Huang, L.H., Wang, C.Z., Kang, L., 2009. Cloning and expression of five heat shock protein genes in relation to cold hardening and development in the leafminer, *Liriomyza sativa*. J. Insect Physiol. 55, 279–285.

Jakob, U., Buchner, J., 1994. Assisting spontaneity: the role of Hsp90 and small Hsps as molecular chaperones. Trends Biochem Sci. 19, 205–211.

Kalleshwaraswamy, C.M., Asokan, R., Swamy, H.M., Maruthi, M.S., Pavithra, H.B., Hegbe, K., Navi, S., Prabhu, S.T., Goergen, G.E., 2018. First report of the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. Pest Manag. Hortic. Ecosyst. 24, 23–29.

Kang, L., Chen, B., Wei, J.-N., Liu, T.-X., 2009. Roles of thermal adaptation and chemical ecology in Liriomyza distribution and control. Annu. Rev. Entomol. 54, 127–145.

Kappe, G., Franck, E., Verschuure, P., Boelens, W.C., Leunissen, J.A., De Jong, W.W., 2003. The human genome encodes 10 *α*-crystalline-related small heat shock proteins: HspB1-10. Cell Stress Chaperones 8, 53–61.

Kim, K.K., Kim, R., Kim, S.H., 1998. Crystal structure of a small heat-shock protein. Nature 394, 595–599.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. Mega X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 356, 1547–1549.

Li, Z.W., Li, X., Yu, Q.Y., Xiang, Z.H., Kishino, H., Zhang, Z., 2009. The small heat shock protein (sHSP) genes in the silkworm, Bombyx mori, and comparative analysis with other insect sHSP genes. BMC Evol. Biol. 9, 1–14.

Lu, K., Chen, X., Liu, W., Zhang, Z., Wang, Y., You, K., Li, Y., Zhang, R., Zhou, Q., 2017. Characterization of heat shock protein 70 transcript from *Nilaparvata lugens* (Stål): its response to temperature and insecticide stresses. Pestic. Biochem. Physiol. 142, 102–110.

Lu, M.X., Hua, J., Cui, Y.D., Du, Y.Z., 2014. Five small heat shock protein genes from *Chilosuppressalis* : characteristics of gene, genomic organization, structural analysis, and transcription profiles. Cell Stress Chaperones 19, 91–104.

Maaroufi, H., Tanguay, R.M., 2013. Analysis and phylogeny of small heat shock proteins from marine viruses and their cyanobacteria host. PloS One 8, e81207.

Morin, M.D., Frigault, J.J., Lyons, P.J., Crapoulet, N., Boquel, S., Storey, K.B., Morin, P.J., 2017. Amplification and quantification of cold- associated microRNAs in the Colorado potato beetle (*Leptinotarsadecemlineata*) agricultural pest. Insect Mol. Biol. 26, 574–583.

Pusag, J.C.A., Jahan, S.H., Lee, K.S., Lee, S., Lee, K.Y., 2012. Upregulation of temperature susceptibility in *Bemisia tabaci* upon acquisition of *Tomato yellow leaf curl virus* (TYLCV). J. Insect Physiol. 58, 1343–1348.

Qin, W., Neal, S.J., Robertson, R.M., Westwood, J.T., Walker, V.K., 2005. Cold hardening and transcriptional change in *Drosophila melanogaster*. Insect Mol. Biol. 14, 607–613.

Rinehart, J.P., Li, A., Yocum, G.D., Robich, R.M., Hayward, S.A., Denlinger, D.L., 2007. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. Proc. Natl. Acad. Sci. Unit. States Am. 104, 11130–11137.

Ritossa, F., 1962. A new puffing pattern induced by a temperature shock and DNP in Drosophila. Experientia 18, 571–573.

Shen, Y., Gu, J., Huang, L.H., Zheng, S.C., Liu, L., Xu, W.H., Kang, L., 2011. Cloning and expression analysis of six small heat shock protein genes in the common cutworm, *Spodoptera litura*. J. Insect Physiol. 57, 908–914.

Shi, M., Wang, Y.N., Zhu, N., Chen, X.X., 2013. Four heat shock protein genes of the endoparasitoid wasp, *Cotesiavestalis*, and their transcriptional profiles in relation to developmental stages and temperature. PloS One 8, e59721.

Sinclair, B.J., Gibbs, A.G., Roberts, S.P., 2007. Gene transcription during exposure to, and recovery from, cold and desiccation stress in Drosophila melanogaster. Insect Mol. Biol. 16, 435–443.

Song, K.H., Jung, S.J., Seo, Y.R., Kang, S.W., Han, S.S., 2006. Identification of up-regulated proteins in the haemolymph of immunized *Bombyx mori* larvae. Comp. Biochem. Physiol. Genom. Proteonomics 1, 260–266.

Sonoda, S., Ashfaq, M., Tsumuki, H., 2006. Cloning and nucleotide sequencing of three heat shock protein genes (hsp90, hsc70, and hsp19.5) from the diamondback moth, *Plutella xylostella* (L.) and their expression in relation to developmental stage and temperature. Arch. Insect Biochem. Physiol. 62, 80–90.

Sun, M., Lu, M.X., Tang, X.T., Du, Y.Z., 2014. Characterization and expression of genes encoding three small heat shock proteins in *Sesamia inferens* (Lepidoptera: Noctuidae). Int. J. Mol. Sci. 15, 23196–23211.

Swamy, H.M., Asokan, R., Kalleshwaraswamy, C.M., Prasad, Y.G., Maruthi, M.S., Shashank, P.R., Devi, N.I., Surakasula, A., Adarsha, S., Srinivas, A., Rao, S., 2018. Prevalence of "R" strain and molecular diversity of fall army worm*Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in India. Indian J. Entomol. 80, 544–553.

Takahashi, K.H., Rako, L., Takano-Shimizu, T., Hoffmann, A.A., Lee, S.F., 2010. Effects of small Hspgenes on developmental stability and micro environmental canalization. BMC Evol. Biol. 10, 284.

Van Montfort, R., Slingsby, C., Vierlingt, E., 2001. Structure and function of the small heat shock protein/α-crystallin family of molecular chaperones. Adv. Protein Chem. 59, 105–156.

Waters, E.R., Aevermann, B.D., Sanders-Reed, Z., 2008. Comparative analysis of the small heat shock proteins in three angiosperm genomes. Cell Stress Chaperones 13, 127–142.

Waters, E.R., Rioflorido, I., 2007. Evolutionary analysis of the small heat shock proteins in five complete algal genomes. J. Mol. Evol. 65, 162–174.

Zhang, L.J., Wang, K.F., Jing, Y.P., Zhuang, H.M., Wu, G., 2015. Identification of heat shock protein genes hsp70s and hsc70 and their associated mRNA expression under heat stress in insecticide-resistant and susceptible diamondback moth, *Plutella xylostella* (Lepidoptera: plutellidae). Eur. J. Entomol. 112, 215–226.

Zhao, L., Jones, W.A., 2012. Expression of heat shock protein genes in insect stress responses. Invertebr. Surviv. J. 9, 93–101.